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HEPARIN-PLASMA AS STOCK-PLASMA FOR TISSUE CULTURES

E. C. CRACIUN

From the Department of Pathology, The Johns Hopkins University, Baltimore

Most tissue culture work is carried out with paraffin-ice-plasma which, however, is not always easily obtainable, especially when the blood of animals with a very short clotting time, such as rats and guinea pigs, is used. Such a plasma must be used at once, in contrast with chick-plasma which, as is well known, may be kept as long as ten to fifteen days.

The rather tedious technique of the ice-plasma, and the large number of animals required to supply the necessary plasma for new cultures and for transfer of old ones, are important factors which prevent a more extensive use of the method for tissue cultures.

Because of these difficulties it seemed desirable, if possible, to develop a less complicated method for preparing pure plasma which might safely be kept in stock for weeks and months.

Such a plasma can be obtained by the use of heparin[1] which is, according to W. H. Howell, a normal constituent of mammalian blood, and plays the rôle of antiprothrombin. Heparin-plasma will clot within fifteen to thirty seconds when put in tissue cultures, because of neutralization of the heparin by the thromboplastic substances which are present in embryonic as well as in adult tissues or, according to Bordet and Gengou, because of the activation of the serozyme-cytozyme reaction.

Howell (7) obtained heparin by extracting dog's liver. He found that 1 mgr. of crude heparin prevents clotting of 5 cc. of cat's blood for twenty-four hours. He demonstrated this by bleeding the animal ten to fifteen minutes after the injection of heparin in quantity proportional to the calculated blood volume. Samples of blood thus obtained, and kept at room temperature, were still unclotted the following day.

[1] Crude heparin may be obtained from Hynson, Westcott and Dunning, Charles Street, Baltimore.

This result was secured by using 1 part of heparin to 5000 parts of blood.

The ratio of heparin to blood can be brought down to 1:10,000 to 20,000 if the blood is drawn from the heart or from a large artery directly into a syringe which already contains a 1:1000 or 2:1000 solution of heparin. The heparinated blood is then poured into a cooled test-tube and placed on ice for three to five minutes; after centrifugalizing, the clear plasma is transferred with a pipette to 1 or 2 cc. ampoules, which are sealed, labeled and kept in a cool place or at room temperature, in the dark. Plasma thus prepared is ready for use at any time, and may be kept for at least seven months according to our experience.

Neither paraffin nor vaseline is necessary in preparing the glassware.

Care must be taken not to mix tissue juice with the blood, and especially lung juice, which contains a great deal of thromboplastic material; otherwise clotting will occur in spite of heparin. In terms of the Bordet theory, the cytozyme from the tissue juice is able to attract the serozyme more strongly than the heparin does and the dispersing action of this antithrombin is thus more readily suppressed (Gratia). Howell (5) showed that heparin has more affinity for thromboplastic material than for prothrombin; the latter is transformed into thrombin when released by heparin, whereupon fibrin develops and a clot is formed. This mixture with tissue juice can be easily avoided by the use of a Lüer syringe. If the needle is in the blood stream, the plunger of this instrument is so light that the blood will be forced into the syringe by its own pressure, and suction is dangerous before the blood is already diffusing into the faintly yellow heparin solution within the syringe, because untimely suction is apt to draw in tissue juice.

Heart puncture with or without opening the thorax is the most efficient method for obtaining blood, especially from young or small animals. The heart is thus allowed virtually to bleed itself into the syringe. The hair is shaved, the skin sterilized and the needle inserted between the ribs at a point 1 cm. above and 1 cm. to the left of the apex of the xiphocostal angle. Thus proceeding, the same animal may be used several times. The opening of the thorax how-

ever avoids much better the mixture with tissue juice and gives a plasma which is stable for a longer time.

A second precaution which is important is that the heparin-blood be cooled immediately upon being drawn. Because of the small quantity of heparin used, neglect of this procedure will result in the coagulation of the plasma within a few days. If it is desired to use embryonal extract in the tissue culture medium, it should be added to the drop of plasma on the coverslip.

Test-tubes, pipettes and ampoules of hard glass should be used. Heparin itself is neutral. There is, however, some danger of getting a plasma with a high pH if it is allowed to remain in contact with a soluble glass. J. H. Brown's method for pH determination has been very helpful in a rather longer series of measurements and would appear especially adapted to tissue cultures work.

Inasmuch as during its preparation heparin is boiled for two hours in alcohol, it can readily be sterilized without any impairment of its activity. The heparin is made up in 8.5 per cent NaCl solution (pH 7.0), sealed in ampoules and then sterilized for fifteen minutes at 15 pounds pressure in the autoclave.

Beside this heparinizing in vitro one can do the same in vivo, that is, to inject first in the jugular vein 1 mg. of the substance for every 5 cc. of the animal's blood, whose total amount is roughly estimated to represent one tenth of the body weight. Ten to twenty minutes later the blood is drawn, cooled and centrifugalized as previously indicated. The plasma obtained in this way is less stabile than that heparinated in vitro.

Mendeleeff found that if embryonic liver be injected intravenously in a guinea pig and the injection repeated after several days the plasma of the animal is distinctly improved as a culture medium. It is not ascertained that heparin, which is a highly purified liver extract, retains the growth promoting action of the fresh extract. Moreover, the intravenous heparin injection being made a few minutes before withdrawing the blood, this change could not take place; at least the pH did not go below 7.4.

The "liver-plasma" of Rhoda Erdmann is prepared in such a way as to contain the growth promoting substances of the fresh liver.

Highly colored plasma will occasionally show a precipitate after twenty-four to forty-eight hours. In order to obtain clear cultures, care should be taken not to disturb this precipitate in removing the plasma from its container.

Such plasma, when contaminated by traces of tissue juice, will develop a tiny meshwork of fibrin. The plasma remains in a fluid state and is still suitable for cultures, although its clotting time, when brought in contact with the tissues, is somewhat prolonged. The clot obtained from such a plasma becomes fluid in a shorter time.

We are inclined to regard this peculiar reaction as similar to the two different stages of clotting described by A. Schmidt in a slowly clotting mammalian blood and by Leo Loeb in the invertebrates. Howell (6) (p. 77) found that mixtures of antithrombin and thrombin (from the dog's blood) may give a protracted clotting with the two stages. A. R. Rich showed that this phenomenon will occur likewise in quickly clotting mammalian blood, if the process is delayed by an appropriate technique, *i.e.*, by very rapid cooling. In our experiment, by the use of heparin, the process seems to be very much delayed, Indeed, the period between the first and second stage appears to be indefinitely prolonged in as far as the second stage does not occur spontaneously.

We have kept heparin-plasma of the rat, guinea pig, rabbit, dog, man and chick for as long as five to seven months. After this lapse of time the plasma clots readily when put into cultures. A drop of plasma, sealed in a hollow-ground slide and incubated at 39°C. without tissue, will still be fluid after weeks.

Tissues grow satisfactorily in heparinated plasma; outgrowth, amoeboid movement, amitosis and mitosis occur to the same extent as for cultures in ice-plasma. This holds for connective-tissue cells as well as for epithelial cells. About six hundred cultures, with and without tissue extracts, were made from heart, kidney, lungs, spleen, ovaries, testicles, thyroid, brain, cornea, skin, amnios and intestine from the species previously mentioned. Embryonic and adult or young tissues were used. Some other twelve hundred cultures, made in ice-plasma, Locke-Lewis, Drew solution and serum, served as controls. These cultures show that cells are not damaged by heparin. No deleterious effects are produced upon the heart beat or upon the

contraction of the extruded muscular cells. This was expected in view of the fact that heparin, being a physiological principle, is to be found constantly in the blood stream (8).

Heparin-plasma will be especially useful to those who work with somewhat limited technical help, and in experiments which require a long period of time.

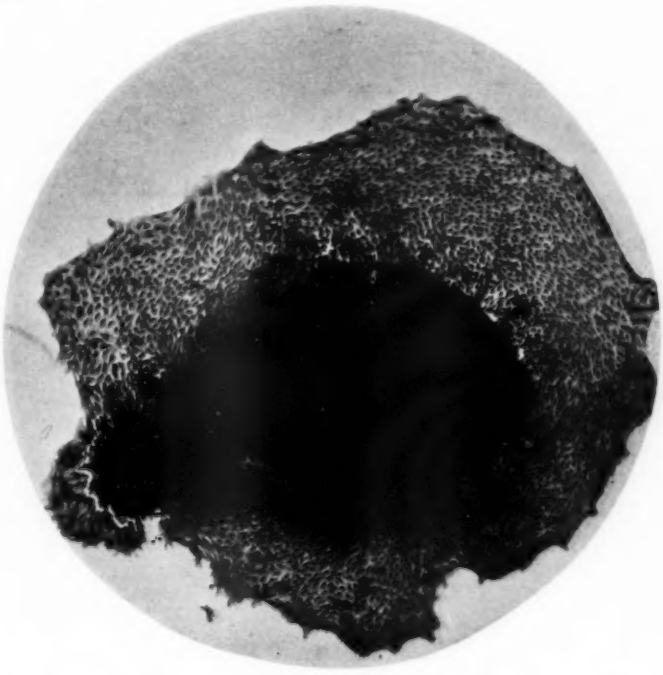

FIG. 1. CULTURE 1367. KIDNEY OF A 16-DAY-OLD RAT IN HEPARIN-PLASMA. FOUR DAYS AFTER THE LAST TRANSFER.

In using the same sample of stock-plasma for many series of cultures, one of the very important factors which control growth in tissue cultures is kept constant. Thus, cultures made at different times may be more easily compared as far as the cell's vitality or the action of extracts, salts, etc., upon growth and outgrowth are concerned.

While citrated or oxalated plasma is no longer pure plasma, heparin (in vitro or in vivo) has proved to meet satisfactorily all theoretical as well as practical requirements for tissue cultures.

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BISMUTH POISONING FOLLOWING ORAL ADMINISTRATION OF BISMUTH SUBNITRATE

WILLIAM H. RESNIK

From the Medical Clinic of the Johns Hopkins Hospital and University

Until the recent introduction of bismuth in the treatment of syphilis, poisoning with this metal was comparatively rare. In 1912, Mayer and Baehr (1) were able to collect 64 cases from the literature, including their own 3 cases; of these 24 were fatal. Relatively few instances were added in the next few years. Practically all of those reported had resulted from the use of bismuth in the treatment of granulating wounds or of chronic suppurative sinuses. Since 1921, however, a number of instances of bismuth poisoning have been recorded, particularly by the French, and, in its minor forms at least, bismuth poisoning is not uncommon in patients to whom it has been given subcutaneously or intravenously as an anti-syphilitic drug. Excellent reviews of this form of poisoning have been published by Mayer and Baehr (1), by Cabot (2), and by Hudelo and Rabut (3). I shall not enter into a detailed discussion of the manifestations of bismuth intoxication, which resembles in many respects that caused by lead or mercury. It will suffice to point out that the most common and important symptoms, the discoloration of the gums and mucous membrane of the mouth, very much like that occurring in lead poisoning and often followed by a more or less severe stomatitis, together with the nephritis and the colitis are primarily dependent on the paths of excretion of bismuth—namely, the salivary and buccal glands, the kidneys and the large intestine. The time of the appearance of the first symptoms depends, of course, on several factors; they have usually occurred in from 10 to 14 days after the use of the drug, although at times they have come on sooner or considerably later.

A smaller number of cases of poisoning have followed the use of bismuth subnitrate given internally, but it has been conclusively shown (1) that in almost all these the intoxication has been due to the nitrite formed in the intestines as a result of reduction of the nitrate. These

cases have been most frequent in children; the symptoms have been acute in onset, occurring within a few hours after the ingestion of the drug and have been characterised by the presence of cyanosis, dyspnea, nausea, and methemoglobinemia. These cases, then, are not to be considered as resulting from the effects of metallic bismuth.

Cohn (6) reported a case of bismuth poisoning in a young woman suffering from pulmonary tuberculosis and "gastric irritability." Following the administration by mouth of about 3 grams of bismuth subnitrate, a blackish discoloration of the gums and parts of the tongue appeared, associated with anorexia and marked prostration. These symptoms disappeared with the withdrawal of bismuth. The case represents the only instance of which I have found any record which may be accepted as one in which *bismuth* poisoning resulted from the administration of this substance by mouth.

There are a few other cases in which the dependence of toxic symptoms following the use of bismuth internally upon metallic bismuth is questionable. Prior's case (7), occurring in an infant following the ingestion of 10 grams of bismuth subnitrate, has been considered by Cabot (2) to be one of bismuth poisoning; on the other hand, Mayer and Baehr (1) have attributed the symptoms to nitrite poisoning, and the latter conclusion would seem more justifiable.

Danulescu and Simici (8) have described an instance in a patient suffering from pyloric obstruction in which acute symptoms, similar to those seen in nitrite poisoning, occurred within two hours after the oral administration of bismuth carbonate, and disappeared in 2 or 3 days. An analysis of the preparation of bismuth showed that it was free from other metals, but that it contained a small amount of soluble bismuth salts; and to these they attribute the symptoms. This case may be a clinical example of the acute form of bismuth poisoning seen experimentally by Steinfeld (4). Since there was no proof, however, that bismuth was actually absorbed, as by the demonstration of bismuth in the urine or by the deposition of bismuth in the gums and buccal mucosa, there must be some question as to whether the symptoms were actually due to systemic bismuth poisoning. These authors cite two other cases from the literature similar to their own, but the same uncertainty exists as to the influence of bismuth in the production of the intoxication.

The following case is described because it represents an undoubted example of systemic poisoning with metallic bismuth following administration of this drug by mouth.

CASE REPORT

B. S., a colored woman of 35, was admitted into the Johns Hopkins hospital on December 21, 1923, complaining of diarrhea and loss of weight and strength. Excepting menstrual irregularities which had been present for a number of years, she had been well until a year before her admission, when she began to have typical symptoms of diabetes mellitus. Later there appeared cramp-like pains in the lower abdomen sometimes associated with vomiting, and periods of intractable diarrhea. Her stools were then thin and watery but never contained blood or mucus.

On physical examination, she showed signs of great loss of weight, exophthalmos (which, she stated, had always been present), oral sepsis, a small adenoma of the thyroid and a large myomatous uterus. Her blood pressure was low (92 systolic, 68 diastolic). Chemical analysis of the blood and urine revealed the findings usually associated with diabetes mellitus. The blood Wassermann was positive. The spinal fluid was normal. The red blood cells were 4,800,000 per cubic millimeter; the hemoglobin was 90 per cent (Sahli); the white blood cells were 6000 per cubic millimeter and the stained smear was entirely normal. The basal metabolism was normal. She responded to the treatment of her diabetes; but the diarrhea persisted in spite of every therapeutic effort, and no adequate cause for its obstinacy was determined after numerous tests and examinations. In a period of five days, she was given a total of 30 grams of bismuth subcarbonate and no untoward symptoms were observed. On January 17, 1924, she was discharged from the hospital, her diarrhea only slightly controlled.

She was treated by her family physician for about a month; because she failed to improve, she was again admitted into the hospital on February 12, 1924. Her symptoms were essentially similar to those present at the first admission, except that in addition there was slight tingling of the legs and feet and slight soreness of the gums. Her physical findings were similar to those described above, and in addition there was an extensive bluish-black stippling of the gums which were swollen and inflamed; there was a similar discoloration of the tongue, occurring at the top of the papillae and arranged in vertical striations along the lateral margins of the tongue. A patchy and more diffusely distributed discoloration of the buccal mucosa was also present, particularly on the left side and about the opening of Stenson's duct. The parotid glands were tender and slightly swollen. There was tenderness over the calf muscles and slight loss in sensation in the legs, feet and hands; there was no muscular weakness. The red blood cells were now 3,100,000 per cubic millimeter, the hemoglobin was 40 per cent, the white blood cells were 9000 and the differential formula was normal. There was distinct basophilic

stippling of the red blood cells, identical with that seen in lead poisoning. A few days after her admission, she had an attack of severe abdominal colic, different from any she had experienced before, and strongly simulating an attack of lead colic.

It was learned from her physician that she had been receiving bismuth subnitrate by mouth and had at no time received lead or any other heavy metal. She had probably taken from 75 to 100 grams of the drug in a period of two weeks before her entrance into the hospital. An analysis of the preparation of the medicine showed that it consisted of bismuth subnitrate, and bismuth was also detected in the urine.

The basophilic stippling of the red blood cells and the tingling of her legs and feet persisted for about a month after her admission into the hospital, the tenderness and swelling of the parotid glands for about two months; and the discoloration of the mouth was still present at the time of her discharge from the hospital on April 12, 1924. At this time the red blood cells were 4,100,000 per cubic millmeter, and the hemoglobin was 60 per cent. During this stay in the hospital there was a partial subsidence of the diarrhea.

She entered the hospital a third time on May 15, 1924, because of the increased severity of her diarrhea. There was still present a faint discoloration of the gums, tongue and the mucosa about the opening of Stenson's duct on the left side. The red blood cells were 4,800,000 per cubic millimeter, and the hemoglobin was 68 per cent. She had had a return of the tingling in her legs and feet. Her condition was practically the same when she left the hospital a month later.

COMMENT

The case described above represents an instance of systemic poisoning by metallic bismuth following the administration of this drug by mouth in the form of the subnitrate. The analyses of the medicinal preparation which the patient had been taking, the demonstration of the presence of bismuth in the urine and the bluish-black discoloration of the mouth justify this conclusion. The stomatitis was similar in its characteristics to that described in most other cases of bismuth poisoning (3, 9). The importance of this symptom lies in the fact that it is the most common and usually the first indication of bismuth poisoning. It usually begins as a bluish-black line about the gingival border of a carious tooth, frequently about the median inferior incisors. As it becomes more extensive, it involves the tongue and buccal mucosa, and later ulcerations may appear, from which Vincent organisms are practically always recoverable.

The present case showed several unusual features. Anemia and basophilic stippling of the red blood cells are generally said to be

lacking in bismuth poisoning, and their absence has been regarded as significant in the differentiation of bismuth from lead poisoning. However, 1 case of bismuth poisoning has been reported in which the red blood cell count fell to 1,600,000 per cubic millimeter (10), and basophilic stippling was seen in 2 cases described by Mayer and Baehr (1). The relationship of the peripheral neuritis in the present instance to bismuth intoxication is questionable, for it recurred some time after bismuth had been omitted (third admission). The affection of the parotids is uncommon; but parotid gland and peripheral nerve involvement have been observed previously, although only on rare occasions (2).

It is apparent that the general picture resembled very closely that due to lead poisoning.

SUMMARY

A case is reported in which bismuth poisoning followed administration of bismuth subnitrate by mouth. The important symptoms were a bluish-black discoloration of the gums, tongue and buccal mucosa, with slight ulceration, moderate anemia, basophilic stippling of the red blood cells, with tenderness and swelling of the parotid glands. There were also abdominal colic and evidences of a mild peripheral neuritis, but the dependence of these symptoms on bismuth poisoning is questionable. The patient recovered.

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ATRETIC FOLLICLES IN THE OVARY OF THE RAT, MOUSE AND RABBIT WITH SPECIAL REFERENCE TO THE SIGNIFICANCE OF THE BASEMENT MEMBRANE IN DETERMINING THE SOURCE OF ORIGIN OF THE INTERSTITIAL CELLS[1]

W. V. WILKERSON

Johns Hopkins Medical School

From the Department of Histology and Embryology, Cornell University, Ithaca, New York; and the Department of Anatomy, Medical College of Virginia, Richmond

INTRODUCTION

An examination of the literature on the ovary shows that atretic follicles have received attention from a large number of workers, either specifically or incidentally, a large part of this study being devoted to some phase of the problem of the interstitial cells.

The interstitial cells appear to have been first described by Pflüger (1863), who found cells in the ovary of the cat that contained fine lipoid granules. Schron, in the same year, described and figured lipoid-containing cells (cat), which he designated as "Stromazellen." His (1865) applied to these cells the term "Kornzellen," described them in detail and discussed their significance. He suggested that they were derived from the spindle-shaped stroma cells or from the blood capillaries with which he considered them to be closely associated. Apparently he did not decide between these two distinctly different modes of origin. Other workers have described a special relationship to the vascular system, but the existence of such a relationship has not gone unchallenged. Waldeyer (1870) considered the "Kornzellen" of His as migratory cells and designated them "Wanderzellen." Tourneaux (1879) compared these cells with the interstitial cells of the testis and applied the same name to them, apparently for the first time. MacLeod (1880), also, used the term "interstitial

[1] Received for publication Nov. 4, 1925.

cells," which has been accepted by all subsequent workers on both the ovary and the testis.

The origin of the interstitial cells has been the subject of controversy from the time they were first described. Some workers have derived them directly from the stroma cells, and hence connective tissue, while others have derived them from the theca interna of atretic follicles, which is only an indirect derivation from the stroma cells. Still other workers have believed that the interstitial cells are epithelial in origin, being derived directly or indirectly from the mesothelial covering of the ovary.

The reader is referred to the paper by Rasmussen (1918) for a discussion of the literature. This author believes that in the woodchuck the interstitial cells which disappear annually are replaced from elements that migrate into the ovary from the germinal epithelium and also by interstitial cells derived from the stroma of the theca interna.

Moore (1921) has derived the interstitial cells from the membrana granulosa. He says: "The end result of follicular atresia is the conversion of the follicular masses into the large-sized cell masses, blackening with osmic acid, which are commonly spoken of as the interstitial cells of the ovary."

MATERIALS AND METHODS

The materials on which this study is based consist of nearly three hundred ovaries of white mice,[2] ranging from embryos of 10 mm. to adults, ninety-two ovaries of the white rat, ranging from birth to old adults, and fifty-eight ovaries of white rabbits of known age, ranging from birth to old adults, and several "half-grown" wild grey rabbits of which the approximate age could be determined with reasonable accuracy.

These ovaries were fixed in Hermann's, Flemming's, Benda's, (Flemming's with one drop of acetic acid to each hundred mils), Carnoy's (6–3–1, with and without the addition of mercuric chloride), Bouin's picro-aceto-formol, and Zenker's (standard formula, and also a modified fluid in which the acetic acid had been reduced to one drop in each 10 mils).

[2] Prepared by Kingery (1917) and used by him as the basis for his study of oögenesis in the mouse.

When animals were sacrificed, the right and left ovaries were treated differently, the most commonly used plan, especially with the rat, being to place one ovary in Flemming's and the other in Zenker's, generally the modified fluid as noted above.

These ovaries were cut from 4 to 10 micra thick and spread as serial sections. Six micra proved to be thin enough for most purposes, although the thinnest sections were necessary for cytological details.

Several methods of staining were employed, among them being Heidenhain's iron hematoxylin, Weigert's copper hematoxylin, Mayer's hematoxylin and eosin, and Bensley's acid fuchsin and methyl-green green staining technique following fixation in his dichromate-acetic-osmic mixture. Iron hematoxylin was used more than any other stain and proved quite satisfactory for most purposes.

Material that had been fixed in Flemming's fluid was either stained with Flemming's triple stain or mounted unstained and without coverglasses. The unstained material proved more satisfactory for this work.

Several ovaries were fixed in the modified Zenker's fluid and then mordanted from one to four weeks in 2.5 per cent potassium dichromate at 38°C. (in an incubator). Ovaries thus treated were stained with iron hematoxylin or with Weigert's copper hematoxylin, as recommended by Kingsbury (1911) for the demonstration of lipoids. This material was particularly satisfactory for bringing out mitochondria.

PRESENT INVESTIGATION

A review of the literature on atretic follicles shows that the work which has been done on the interstitial cells falls under four general heads: their origin, their presence and relative abundance in the adults of different species, their morphological alterations as related to the sexual cycle, and the question of the interstitial cells as producers of an internal secretion. In the present study we shall refer only to the first and last of these four phases.

In this paper the term "interstitial cells" will be applied to those cells of relatively large size, situated between the follicles in the connective-tissue stroma, with spherical or nearly spherical nuclei and with cytoplasm that contains fine granules which blacken after fixation in osmic acid mixtures.

It is, of course, well known that the normal theca is formed from the stroma cells. During the development of follicles, from their primary resting stage to the many-layered Graafian follicle, the cells of the theca become progressively more distinct, although they retain the spindle shape and general histological characteristics of the stroma cells. They soon become differentiated into a vascular theca interna, characterized by large cells with abundant cytoplasm, and a less vascular theca externa, having smaller cells which generally appear more slender than those of the interna.

The theca and the membrana granulosa are separated by a basement membrane which can sometimes be seen quite distinctly.

The normal ovary probably always contains a great many more growing follicles than are required to supply the comparatively small number of ova which escape from mature follicles. These surplus follicles are overtaken in the course of their development by follicular atresia.

The cells of the membrana granulosa of atretic follicles are generally the first to show degenerative changes. The cell boundaries early disappear and the cytoplasm becomes so changed that it stains more deeply than formerly, losing its finely reticulated appearance. Fat droplets may also appear in the cytoplasm, although normal follicle cells show little or no free lipoids. These changes in the cytoplasm are accompanied by profound degenerative changes in the nuclei. As the nuclei degenerate, the chromatin becomes condensed into a single irregular mass, or in some cases arranges itself into several knots, which may sometimes be seen lying free in the cytoplasm after the nuclear membrane has disappeared. This is evidently the process of chromatolysis (karyorrhexis) early described by Flemming. The end-result of these nuclear and cytoplasmic changes in the follicular epithelium is the liquefaction and final disappearance of all cells affected.

The rows of cells bordering the follicular cavity are the first to be affected by these degenerative processes (Fig. 2). This may be due to the fact that they are farther removed from the nutritive supply than the layers nearest the basement membrane. Changes in the neighboring cells soon follow, the cells nearest the basement mem-

brane being the last to lose their normal appearance. In a large number of follicles the cells nearest the antrum were found to have undergone marked cytoplasmic changes, while the cells nearest the basement membrane appeared still to be perfectly normal. Numerous sections have likewise been examined in which a few layers of degenerating cells, sometimes parts of one or two layers only, were present near the basement membrane, the remaining layers having disappeared. This progressive degeneration from the cavity of the follicle towards the basement membrane is not so characteristic in

FIG. 1

FIG. 2

FIG. 1. Rat, 47 days. The ovum is flattened and the egg-bearing hillock shows degenerative changes. Modified Zenker's, iron hematoxylin stain. × 300.

FIG. 2. Rat, fully mature. The egg-bearing hillock is breaking down, allowing the ovum to escape into the antrum of the follicle. Degenerative changes are also appearing in the cells of the stratum granulosum. Modified Zenker's, iron hematoxylin stain. × 300.

the rabbit as it is in the rat and mouse, for we very often find follicles in which nearly every cell of the stratum granulosum is undergoing profound alterations (Fig. 10). In these cases it is common to find that the degenerating cells have broken loose from their usual position and are to be found in an irregular mass in the follicular cavity.

Eventually all the cells of the membrana granulosa degenerate and disappear, leaving the theca, now mostly interstitial cells, separated from the antrum of the follicle by the basement membrane, which is often, perhaps generally, unbroken and quite distinct.

The cumulus oöphorus breaks down quite early, allowing the ovum to escape into the antrum of the follicle. It is not uncommon for

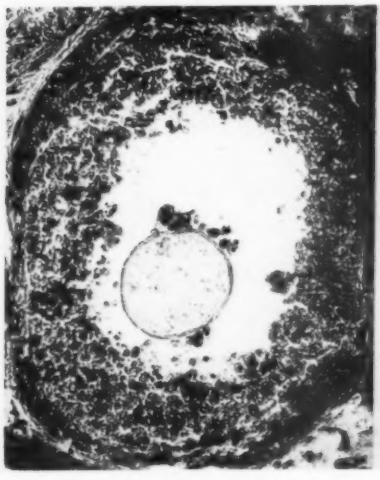


FIG. 3

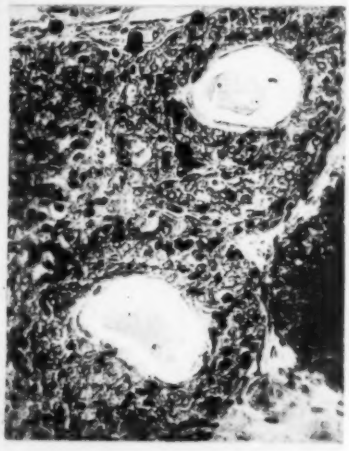


FIG. 4

FIG. 3. Rat, 32 days. The egg-bearing hillock has degenerated so that the ovum is free in the antrum of the follicle, with a portion of the hillock adhering to the ovum. Degenerative changes in the stratum granulosum are evident. Modified Zenker's, iron hematoxylin stain. × 300.

FIG. 4. Rat, 47 days. The membrana granulosa has completely disappeared leaving the theca, now mostly interstitial cells, separated from the antrum of the follicle by an unbroken basement membrane. Modified Zenker's, iron hematoxylin stain. × 360.

one or more layers of cells to adhere to the ovum when it passes into the follicular cavity (Fig. 3). These cells, however, soon degenerate and disappear.

The ovum itself degenerates more slowly than the membrana granulosa. In undergoing degeneration the cytoplasm of the egg

cell becomes so changed that it stains more deeply than formerly and appears denser and hyaline in character, the finely reticulated appearance having been lost. Quite frequently the ovum disappears in the cavity of the follicle without undergoing any marked changes, the nucleus becoming more or less homogeneous.

The egg cell may also disappear by a process of degenerative fragmentation. This process, as found in the mouse, has been described at length by Kingery (1914). Such fragmentation occurs most frequently during the period just preceding sexual maturity, when follicular atresia is greatest, although it has been observed in adult

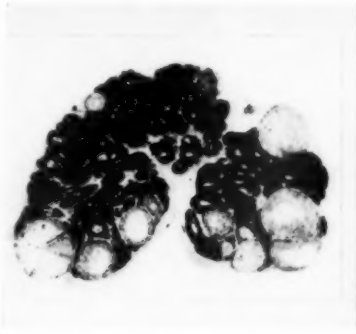


FIG. 5

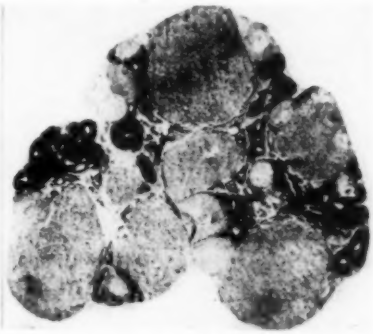


FIG. 6

FIG. 5. Rat, 47 days. Section through ovary fixed in Flemming's. Note abundance of interstitial cells, which appear as blackened masses. No stain, no cover-glass. × 25.

FIG. 6. Rat, 74 days. Corpora lutea are present and interstitial cells less abundant than in the section shown in Fig. 5. Fixed in Flemming's, no stain, no cover-glass. × 25.

animals. This phenomenon was noted most frequently in the rat and mouse, being rather uncommon in the rabbit.

During the process of fragmentation the ovum becomes divided into two or more portions, one section of my material (rat) showing eleven of these fragmentary "cells." Follicles in which the ovum has become broken up into from four to six portions are quite common (Fig. 8). Most of these "cells," although not all of them, have chromatin material simulating nuclei, probably derived from the original

nucleus of the oöcyte. Some of these segments have several such pseudo-nuclei. This chromatin is often found arranged in small granules around the outer part of the vesicle in which it appears, although larger masses are sometimes seen, being presumably formed by the adhesion or coalescence of the finer granules.

After a time all these fragmentary "cells" degenerate and disappear. Extra-ovular cells that penetrate the zona pellucida may play an important rôle in their disappearance. These extra-ovular cells are found embedded in the cytoplasm of the fragments of the ovum, between these fragments, and along the inner border of the zona pellucida. They are not to be confused with the pseudo-nuclei of the fragmentary "cells," for they are structurally different, containing fine granules that stain an intense black with iron hematoxylin. Such cells may often be seen inside the zona pellucida for some time after the egg cell itself has completely disappeared.

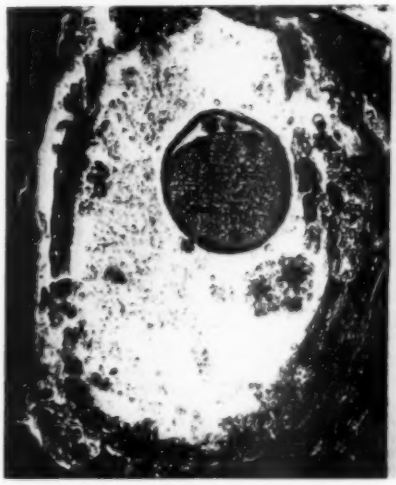
It seems highly probable that these extra-ovular cells have a definite rôle in connection with the disappearance of the egg cell, for they are found not only associated with the ova which degenerate by fragmentation but also with those which do not undergo fragmentation. It has been suggested that their action is one of phagocytosis, and it is most probable that they do possess phagocytic properties. They are also sometimes seen in the cytoplasm surrounded by clear spaces, and the suggestion is therefore close that these cells may elaborate some chemical substance which is responsible for the liquefaction and perhaps absorption of the ovum.

As the granulosa degenerates, the theca interna hypertrophies and the basement membrane contracts, thus encroaching upon the antrum of the follicle which becomes progressively smaller. This process is probably facilitated by the resorption of the follicular contents within the basement membrane. As the membrane contracts, it also becomes thicker and is, therefore, much more easily seen than in normal follicles.

While the structures within the basement membrane are undergoing the changes already described, the cells of the theca, particularly the theca interna, are undergoing marked changes. The spindle-shaped cells of the theca become transformed into large polyhedral cells with relatively clear vesicular nuclei and an abundance of lipoid-

laden cytoplasm. Intermediate stages between the spindle-shaped theca cells and the large polyhedral cells are usually abundant. These large polyhedral cells blacken after fixation in osmic acid mixtures and thus meet all the conditions requisite for classification as interstitial cells.

Limon (1902) believed that, "Dans la region periphérique, les cellules sont agglomeriés en amas compacts de configuration assez uniforme" (rat). He described this configuration as consisting of



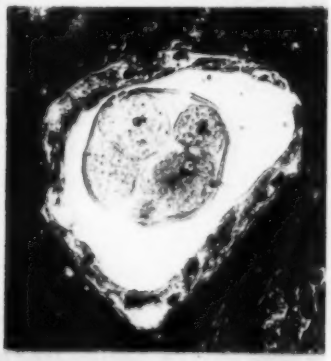


FIG. 7 FIG 8

FIG. 7. Rat, 32 days. Note the two polar bodies within the zona pellucida, while the ovum is still within the ovary. Modified Zenker's, iron hematoxylin stain. × 300.

FIG. 8. Rat, 38 days. This section shows a "five-cell stage" in the degenerative fragmentation of the ovum as sometimes seen in atresia folliculi. Modified Zenker's, iron hematoxylin stain, × 300.

cords of cells arranged perpendicularly to the surface of the ovary. This observation, apparently, has not been confirmed by subsequent workers. In my material there certainly is *not* any such orderly arrangement of cords of cells, the interstitial cells being found as isolated cells or more frequently as irregular masses of cells scattered

indiscriminately throughout the ovary, their position quite evidently being determined by the stress of growth changes in the ovary.

There is sometimes a very thin layer of cells in direct contact with the basement membrane that does not become transformed into interstitial cells, apparently returning directly to the stroma cell type. The basement membrane is frequently quite distinct and is often, perhaps generally, unbroken. The fact that the interstitial cells are found grouped around the *outside* of an unbroken basement membrane renders it highly improbable that they could have been formed from the follicular epithelium (membrana granulosa), even if the degeneration and disappearance of the granulosa were not so easily demonstrated, for we can hardly expect that such a large number of cells could pass through the membrane and leave it intact (Figs. 4 and 11).

In the rat and mouse the presence of a very small number of interstitial cells is first evident about two weeks after birth. Limon (1902) reports finding them in ovaries of rats of eleven days. At this early age there are only a very few interstitial cells present, their formation not becoming very pronounced until about the third week. Atretic follicles, and hence interstitial cells, are constantly present from this time on, becoming progressively more abundant as the animal approaches sexual maturity. Atresia folliculi is most marked during the prepubertal period, the ovary becoming packed with irregular rings of interstitial cells (Fig. 5). These cell masses gradually break up and the interstitial cells become scattered throughout the ovary, apparently indiscriminately, in poorly defined groups.

The number of interstitial cells is greatest, in the rat and mouse, at the beginning of sexual maturity. Owing to the appearance of corpora lutea they are apparently very much reduced in number in the adult animal, but quantitative studies by Osterud (23) show that the decrease is largely relative rather than actual, although a progressive actual decrease is present, the total volume of interstitial cells decreasing slowly.

In the rabbit the interstitial cells were first recognized in animals seven weeks old and were not always present at this age. They become progressively more abundant as the animal approaches adult life, the degeneration of follicles being most pronounced from the third to the sixth month. Interstitial cells are most abundant in mature animals, where the ovaries appear to be largely made up of such cells.

The writer fully agrees with Sainmont (1905) and Kingsbury (1914) that the interstitial cells revert to their original stroma-cell form, the process apparently being one of de-differentiation. Intermediate stages in the transformation can generally be found without difficulty. It is, of course, possible to interpret these intermediate stages as theca cells that have failed to be completely transformed into inter-

FIG. 9

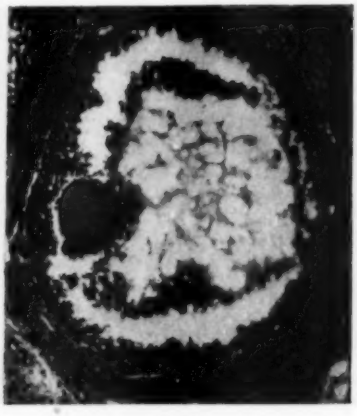


FIG. 10

FIG. 9. Rabbit, five months. The membrana granulosa is beginning to show pronounced evidence of degeneration. Note the loose arrangement of the cells and the numerous fine but distinct granules, which may be seen throughout the membrana granulosa, particularly near the antrum. Modified Zenker's, iron hematoxylin stain. × 170.

FIG. 10. Rabbit, five months. Note the marked degeneration of the membrana granulosa. It will be seen that most of the cells have lost their normal appearance, the fine granular appearance described in Fig. 9 being conspicuous in this section. The cells of the granulosa have become separated, forming an irregular mass in the antrum of the follicle. Modified Zenker's, iron hematoxylin stain. × 170.

stitial cells, or as stroma cells in the process of conversion from the stroma-cell type into interstitial cells. It seems more probable, however, that they represent stages in the reversion (de-differentiation) of interstitial cells to the stroma-cell type.

If these intermediate stages represent theca cells that have failed to become completely transformed into interstitial cells, we should expect to find them fully as numerous in groups of newly formed interstitial cells as in groups that have obviously been present for a longer time. This does not appear to be the case. We find very few of the intermediate stages in the recently formed cell groups, whereas they are often quite numerous in the older cell groups. This fact would

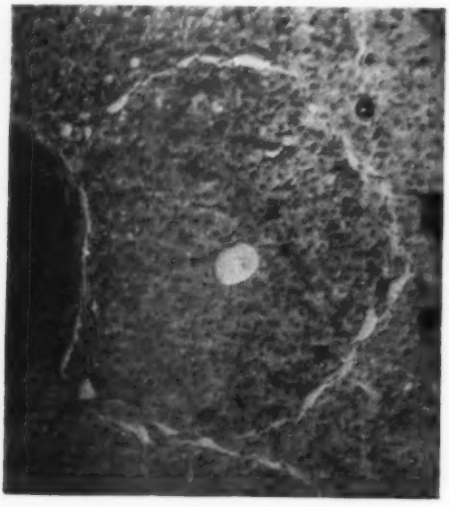


FIG. 11. RABBIT, SIX MONTHS

The membrana granulosa has completely disappeared, leaving the theca, now interstitial cells separated from the antrum of the follicle by an unbroken basement membrane. Modified Zenker's, iron hematoxylin stain. × 170.

seem to exclude their interpretation as incompletely formed interstitial cells.

If the intermediate stages are stroma cells becoming transformed directly into interstitial cells, we should expect not only to find them throughout the stroma as well as in the interstitital cell groups, but should naturally expect to find them there in greater numbers because of the greater number of stroma cells in any given area and

hence greater numbers of potential intermediate stages. Any careful study will show that this is not the case but that they are found in much greater abundance among old interstitial cell groups. For the reasons given above, the intermediate stages between the stroma-cell type and typical interstitial cells are not considered as imperfectly developed interstitial cells or stroma cells that are being transformed directly into interstitial cells, but are believed to represent stages in the reversion of interstitial cells back to the stroma-cell type from which they were originally derived.

The rather abundant material at my disposal fails to show any evidence supporting the opinions of Limon (1902), Sainmont (1905), Aime (1907), Athias (1911), and earlier workers, that the interstitial cells bear a special or peculiarly close relationship to the vascular system. It is well known that the theca of atretic follicles is much less vascular than that of normal follicles. If the interstitial cells possess any function that requires a special relationship to the blood capillaries and lymphatics, it seems reasonable to assume that the vascular supply would not be diminished just at the time that the cells are developing and would be expected to begin to function in their special rôle.

DISCUSSION

In the preceding description of conditions as found in the ovaries of the rat, mouse, and rabbit, it was pointed out that the interstitial cells were formed as a modification of the cells of the theca interna of atretic follicles. The writer wishes to emphasize the fact that in a very large percentage of these cases the developing interstitial cells were separated from the membrana granulosa and the cavity of the follicle by an unbroken basement membrane. Large numbers of follicles in the later stages of atresia have been examined in which the granulosa cells have completely disappeared, leaving a well defined and unbroken basement membrane surrounded by a mass of unmistakable interstitial cells. It is difficult to understand how this relationship can be explained if the interstitial cells are derived from the cells of the stratum granulosum. Since we do so often find an unbroken basement membrane, it seems reasonable to assume that the interstitial cells, if they are derived from the follicle cells, would frequently be

found *inside* the membrane. In no instance was this the case. It is hardly to be expected that such large numbers of cells would pass through the membrane without destroying it, hence we much conclude that they are formed *outside* the membrane (from the theca interna of atretic follicles), the follicle cells degenerating and disappearing.

A satisfactory interpretation of the interstitial cells is made difficult by their position which is such as to make it extremely hard, if not indeed impossible with our present facilities, to secure definite experimental evidence upon which to base our conclusions. In the absence of such experimental evidence it is more or less futile to speculate as to their significance.

It seems highly probable, basing my opinion upon the data afforded by this study, that the increase in size of the theca cells, the increase in cytoplasm and the nuclear changes, as well as the appearance of lipoid granules in the theca cells, are caused by the mechanical and nutritive changes within the ovary that are responsible for atresia of the follicles. The interstitial cells would thus stand as an expression of altered mechanical conditions and an altered metabolism of the follicle, and are believed to pass through the period of their existence as interstitial cells and to revert to the stroma-cell type from which they were derived without any ulterior significance as producers of an internal secretion.

The writer is indebted to Professor B. F. Kingsbury, Cornell University, under whom the greater part of this study was conducted, for generous assistance and guidance, and to Professor H. L. Osterud, Medical College of Virginia, for numerous helpful suggestions.

CONCLUSIONS

1. The interstitial cells are formed from the theca, particularly the theca interna, of atretic follicles and are, therefore, of connective-tissue origin.

2. In the rat and mouse the interstitial cells are found as irregular groups scattered throughout the ovary and do not appear to have any definite arrangement, their position apparently being determined by the stress of growth changes within the ovary.

In the adult rabbit the ovary is packed with interstitial cells, but these are not believed to be arranged in any special order or according to some definite plan.

3. In the rat and mouse the interstitial cells appear about the second week after birth, with individual variations, and are constantly present from this time on. They appear quite suddenly, becoming progressively more abundant as the animal approaches sexual maturity. Their number becomes much reduced after the period just preceding sexual maturity has passed.

The interstitial cells were first definitely recognized in the rabbit in animals seven weeks old. They are constantly present from this time on, being most abundant in the adult animal.

4. The oöcyte may degenerate without any marked changes, or it may undergo degenerative fragmentation, becoming broken up into numerous portions that have chromatin-containing nuclei and these fragmentary cells disappearing. There is an unquestionable invasion of extra-ovular cells which are presumably connected in some way, possibly as phagocytes, with the final disappearance of the oöcyte.

Fragmentation was not noted often in the rabbit, the process of degeneration being otherwise as in the rat and mouse.

5. A special or peculiarly close relationship to the blood capillaries or to the lymph vascular system could not be demonstrated.

6. The interstitial cells are not believed to constitute a gland of internal secretion.

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THE OCCURRENCE OF CHORIOANGIOFIBROMA (CHORIOANGIOMA)

A Study of Six Hundred Placentas

R. S. SIDDALL

From the Department of Obstetrics, Henry Ford Hospital, Detroit, Michigan

A careful bibliographical research included in a recent study of angiofibroma of the chorion (1) revealed only 131 authentic cases since the first report by John Clarke in 1798, indicating that these tumors occur very infrequently. This rarity has been noted repeatedly and, although it has been suggested that probably many tumors are overlooked, Leopold (2) found only one such growth in 7,000 to 8,000 placentas and Schickelé (3) two in 20,000. On the other hand, Kraus (4) saw four examples in one year at the First Vienna Clinic and believed that many small tumors were not reported. Brindeau and Natten-Larrier (5) reported only four in six years from different hospitals of Paris, but stated that they had not infrequently found small masses resembling the large tumors in structure. During the routine microscopic examination of placentas, from which only one or two pieces were taken, it has been our experience that small angiofibromata are occasionally found. Consequently, their apparent rarity seemed questionable, and, while placentas were being carefully studied for another purpose, a search was also made for these tumors. Definite angiofibromata were found in six placentas out of six hundred.

The material and method of examination were as follows: Six hundred placentas from consecutive deliveries, the majority at term and none before the eighth lunar month of pregnancy, were weighed, measured, and examined closely. They were then hardened in ten per cent formalin solution (four per cent formaldehyde) for three to eight weeks and sectioned in slices five to eight millimeters thick. All interesting objects such as infarcts, cysts, and tumors were de-

scribed and later, together with a section of umbilical cord, were examined microscopically.

CASE REPORTS

Case 1. E. R., American, white, aged 34, seventh pregnancy. The history and physical examination were negative; blood Wassermann negative with plain, cholesterinized, and Kolmer antigens; pelvis normal. Pregnancy was uneventful—no hydramnios or albuminuria, blood pressure never above 115 systolic and 75 diastolic. Labor was spontaneous at term—duration 1½ hours, presentation vertex. The placenta separated early and was expressed from the vagina. Blood loss 100 cc. The child was normal, weighed 4100 grams, and was 53 cm. in length. The puerperium was afebrile, and the mother and child were in excellent condition when discharged on the fourteenth day.

Gross examination of the placenta: There is nothing noteworthy externally; weight 710 grams; dimensions 20 by 16.5 by 1.5 cm. The umbilical cord appears normal, is 70 cm. long, and is inserted directly and eccentrically. Sections of the placenta reveal nothing worthy of note, and one block and a section of umbilical cord are cut for microscopic examination.

Microscopic examination: A cluster of seven angiomatous villi are seen about 2 mm. from the decidual surface. The largest is irregularly oval, being about one millimeter in length and slightly less in width. One end is adjacent to, but does not fuse with, a decidual (or trophoblastic) septum. It is partially divided into two lobes by a double layer of syncytial epithelium extending inward from the covering layer. The other bodies vary in size but are all much smaller. They are close to, or directly against, the larger mass from which three seem to be actual outgrowths. This picture suggests that we are dealing with a lobed or arborescent type of tumor, which is made up of a main mass with angiomatous outgrowths. The microscopic structure of the bodies is the same, consisting of numerous blood-filled capillaries lined with a single layer of endothelium and separated by narrow septa of loose, sparsely nucleated, fibrous tissue. The capillaries in general are about as large as those in normal villi, though a few are several times this size. The covering layer of epithelium is composed of well preserved syncytium lying close to the outer capillaries, the intervening connective-tissue layer being no thicker than that between adjacent capillaries. Otherwise, the placenta shows nothing worthy of note except a small white infarct beneath the fetal surface The umbilical cord is normal.

Case 2. F. C., American, white, aged 27, first pregnancy. There was nothing noteworthy in the history, and the physical examination was essentially negative—pelvis normal, blood Wassermann negative with Kolmer antigen. Pregnancy was normal—no hydramnios or albuminuria, blood pressure never above 125 systolic and 75 diastolic. Spontaneous labor occurred at term—duration 25½ hours, position L. O. A. The placenta separated rapidly and was expressed

from the vagina. Blood loss 200 cc. The child was normal, weight 2955 grams, length 50.5 cm. The puerperium was afebrile, and mother and child were in excellent condition when discharged on the seventeenth day.

Gross examination of the placenta: Externally there is no abnormality. The weight is 500 grams, and the dimensions are 18 by 17 by 2.5 cm. The umbilical

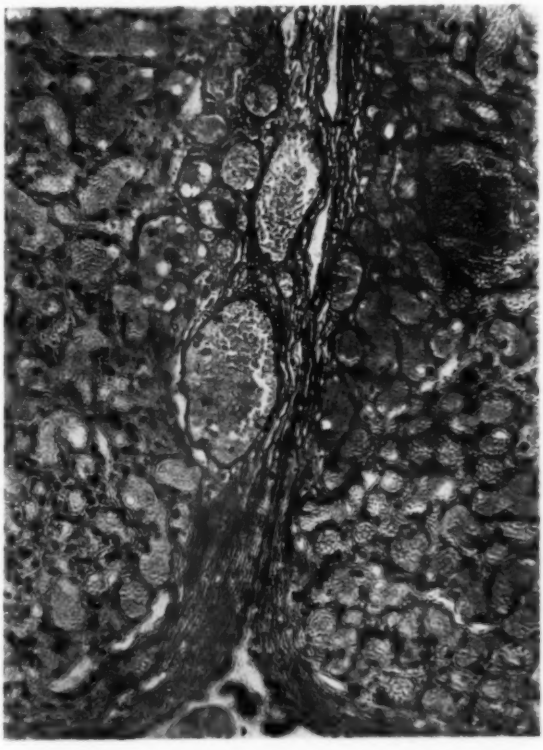

FIG. 1. FROM ONE OF THE TUMORS OF CASE 2, SHOWING CLOSE APPOSITION OF LOBES

The tissue of one lobe is very cellular, while that of the other is fibrous

cord is 53 cm. long and is inserted directly and centrally on the placenta. Sections show a striated dark brown body, 8 mm. in diameter, adjacent to the maternal surface, and two well defined, nearly round, white objects, 7 mm. in diameter, one being just under the chorionic layer and the other beneath the first and separated from it by a narrow line of placental tissue.

Microscopical examination: The two white masses are found to be chorioangiofibromata and show essentially the same structure. The epithelial covering is well preserved and consists of single-layered syncytium, beneath which, for most of the circumference, there are one to several rows of what appear to be Langhans' cells. The surrounding villi are normal except that some are undergoing white infarction in several narrow areas adjacent to the tumor. Beneath the epithelial covering there is a narrow layer of rather compact, but cell-poor, connective tissue, from which narrow septa, containing many small arteries and veins, pass inward and partially mark off seven lobes in one tumor and four in the other. In the latter, the epithelium at one place invaginates so as nearly to bisect the tumor (Fig. 1). Smaller septa branch off from the main ones and incompletely divide the lobes into small lobules whose stroma is made up of dense connective tissue and scattered blood-filled capillaries of the usual size. In many places the capillaries are few, and the connective tissue predominates. This tissue is very cellular and at first glance suggests sarcoma because of marked variation in size and shape of the cells—spindle, oval, and round. However, a careful search reveals no mitotic figures. The striated dark area has the usual structure of the so-called red infarct. The umbilical cord is normal.

Case 3. L. M., American, white, aged 37, first pregnancy. History and physical examination negative; pelvis normal; blood Wassermann negative with Kolmer antigen. Pregnancy was complicated by Bacillus coli pyelitis of the right kidney which cleared up promptly with ureteral catherization and irrigation of the kidney pelvis. The blood pressure never exceeded 135 systolic and 90 diastolic, and there was no hydramnios. Spontaneous delivery occurred twenty days before the calculated expected date of confinement after a labor of 15¾ hours. The placenta separated early and was expressed from the vagina. Position was L. O. A., blood loss 400 cc. The child was small but otherwise normal. Weight 2290 grams, total length 45.5 cm., crown-rump length 31 cm. The puerperium was afebrile, and mother and child were in excellent condition at discharge on the eighteenth day.

Gross examination of the placenta: Externally there is nothing unusual. The weight is 435 grams; dimensions 14 by 13 by 3 cm. The umbilical cord is 60 cm. long and is inserted directly and eccentrically. Sections of the placenta show three chorioangiofibromata. The largest, which is against the decidual surface, is nearly round and measures 1.5 cm. in diameter. It is sharply defined, but there is no capsule formation. The mass is firmer than the normal, spongy, placental tissue, is somewhat darker, and has the appearance of being composed of more compact tissue. There is one septum of lighter tissue in which are several blood vessels. Another slightly smaller mass of the same general appearance is situated just beneath the amniotic surface to one side of the insertion of the umbilical cord. There are no septa, but several comparatively large blood vessels enter the mass at the deepest-lying portion. The third tumor, which is about a centi-

meter from the placental margin and 3 mm. beneath the amniotic surface, is oval in shape and measures 12 by 7 mm., the greater diameter being parallel with the placental surface. This mass is marked off by a narrow, grayish capsule. It is lighter than the other tumors, appearing to be more fibrous. Several septa divide it into lobes. Nothing further of interest is seen.

Microscopical examination: The first tumor is near, but not against, the decidua and consists of a solitary body without any tendency toward lobulation, except for the partial septum mentioned before, through which small vessels enter the tumor from its base on the deepest (upper) part of the periphery. Otherwise, the internal structure resembles that of the second mass, which is just under the chorion and consists of a main and four smaller lobes which are pressed together so closely as to give the gross appearance of one mass. The structure of all the bodies is the same; and although no direct connection is seen at the place of examination, no doubt the small masses are outgrowths from the large body, the tumor having developed by a lobular or arborescent type of growth. With higher magnification, numerous small bud-like nodules attached to the larger masses are visible. Apparently the pressure of expansive growth has been considerable, as the tumor masses have facetted sides, and in many places the single-layered syncytial covering is degenerating and has even disappeared at some points. The villi nearby do not show so much evidence of pressure. Some are undergoing degeneration similar to that usually described as infarct formation, but this process is no more marked here than throughout the placenta. Beneath the syncytial covering of each mass there is generally a thin layer of scantily-nucleated connective tissue, but in some places this is entirely lacking, and epithelium is directly against the stroma. Except for a few arteries and veins in the largest mass, the internal structure of all the nodules consists of numerous blood-filled capillaries separated by rather loose and fairly cellular, areolar tissue resembling that of normal villi. The capillaries have single-layered endothelial walls and in general are similar to those found in normal villi.

The third tumor has a different structure. It is surrounded by a narrow layer of degenerated villi plastered together by fibrin so as to form a complete pseudocapsule against the epithelial covering of the tumor. This covering consists of several layers of vacuolated, irregularly round or oval cells with large, pyknotic nuclei—the usual picture of epithelial remains found in areas of white necrosis or infarction. Many cells are undergoing degeneration, and in a few places the whole layer is represented by shadows of cells with fragmented nuclei. Within this covering there is a narrow layer of loose, areolar tissue which in some places is almost myxomatous in appearance. Nuclei are few and are oval or spindle-shaped with the long axis parallel to the tumor surface. At several points the nuclei are disappearing, and hyaline degenerative changes are beginning. From this layer many septa of similar appearance extend inward, sometimes entirely across the tumor, dividing it into lobes and then by numerous branches into small lobules. In the septa there are small arteries and veins. The stroma of the

lobules consists of numerous capillaries separated by fairly cellular connective tissue with oval and spindle-shaped nuclei. The vessels in a few places appear normal, but usually their endothelial walls are degenerating or have entirely disappeared. They are filled with changed blood whose red cells in many areas no longer take the stain but are represented by faint shadows or fragments of cells. In some places the connective tissue is beginning to degenerate, but nowhere does it show the same degree of change as the capillaries. Evidently, the nutrition of the tumor has been interferred with, and the entire mass is undergoing degeneration and necrosis. Except for these tumors the placenta shows nothing noteworthy, and the umbilical cord is normal.

Case 4. L. I., American, white, aged 29, third pregnancy. The history and physical examination were negative. The pelvis was normal, and blood Wassermann negative with Kolmer antigen. Pregnancy was uneventful, no albuminuria or hydramnios; highest blood pressure 110 systolic and 70 diastolic. Delivery was spontaneous at term after a labor of 6¼ hours. Position L. S. A. The placenta separated early and was expressed from the vagina. Blood loss 50 cc. The child was normal, weight 3540 grams, length 51 cm. The puerperium was afebrile, and mother and child were in excellent condition when discharged on the sixteenth day.

Gross examination of the placenta: Nothing unusual is made out externally. The placenta weighs 665 grams and measures 21 by 18 by 3 cm. The umbilical cord is 56 cm. long and is inserted directly and eccentrically. Sections show only one abnormality—a small, slightly oval chorioangiofibroma which measures 12 by 10 mm. and is situated 2 cm. from the margin and about 2 mm. beneath the fetal surface. It is darker and somewhat firmer than the surrounding tissue and sharply demarcated from it, but there is no capsule. Except for several small blood vessels the interior of the tumor appears homogeneous, and no septa are seen.

Microscopical examination: The tumor has a complete epithelial covering of single-layered syncytium beneath which are occasional short rows of what appear to be Langhans' cells. In many places, villi lie against and indent the periphery of the tumor, but nowhere is there fusion. Some of the adjacent villi are undergoing necrosis similar to the so-called infarct development, but not yet to the point of capsule or pseudo-capsule formation. For about half of the circumference there is a thin layer of compact connective tissue within the epithelial covering, but there are no trabeculae extending inward, and except for a few small arteries and veins the tumor consists of blood-filled capillaries separated by loose connective tissue. The capillaries are about the size of those in normal villi, and their walls consist of a single layer of endothelium. They are distributed fairly uniformly throughout the tumor and in general are about their own width apart. The intervening connective tissue resembles that of normal villi. In fact, except for somewhat more numerous capillaries, the internal structure of

the tumor is identical with that of normal villi. The placental tissue elsewhere is normal, and the umbilical cord shows nothing worthy of note.

Case 5. G. W., American, white, aged 26, first pregnancy. History and physical examination were negative, The pelvis was normal, and blood Wassermann

FIG. 2. FROM CASE 5, SHOWING CLOSE APPOSITION OF TWO LOBES, THEIR SYNCYTIAL COVERINGS BEING PRESSED TOGETHER

On either side is seen the capillary angiomatous stroma

negative with Kolmer antigen. Pregnancy was normal except for moderate but persistent hypertension of 160 systolic and 110 diastolic. No albuminuria or hydramnios. Onset of spontaneous labor occurred at term four hours after premature rupture of the membranes. The placenta separated early and was expressed from the vagina. Duration of labor 14¾ hours; position R. O. P.; blood loss

525 cc. The child was normal; weight 3680 grams, length 52 cm. Except for a mild puerperal infection the puerperium was uneventful, and mother and child were in excellent condition when discharged on the fourteenth day.

Gross examination of the placenta: Externally the placenta is normal; the weight is 725 grams and the dimensions are 18.5 by 16 by 2.5 cm. The umbilical cord is normal and is 86 cm. long. Sections of the placenta show a round chorioangiofibroma, 8 mm. in diameter, just beneath the amniotic surface near the center of the organ. It is sharply demarcated and is darker and firmer than the normal, spongy, placental tissue. There is nothing further of interest except a small cyst.

Microscopical examination: The tumor consists of three lobes of about the same size, which, although closely pressed together, are each covered by a single to several-layered syncytium (Fig. 2) beneath which at some points are a few Langhans' cells. Within this investment there is a layer of compact but cell-poor connective tissue. Near the upper poles the epithelium disappears, and the lobes are joined as a cluster to a blood vessel, from which branches enter the various parts of the tumor. Connective tissue passes inward with the vessels and divides the lobes incompletely into lobules. The stroma of the lobules consists of many blood-filled capillaries separated by narrow septa of fairly cellular, fibrous tissue; and, except for more numerous capillaries, the internal structure does not differ essentially from that of normal villi. In one lobe early necrotic changes are taking place, especially at the lower pole away from the hilus. The blood cells here are indistinctly outlined and stain faintly, and the endothelium of the capillaries is beginning to degenerate. Evidently there has been some disturbance of circulation, though there is no evidence of this in the hilus vessels. The placenta elsewhere is essentially normal except for a small chorionic cyst and for necrosis of the villi in the narrow space between the tumor and the chorion. The umbilical cord is normal.

Case 6. U. B., American, white, aged 23, first pregnancy. History and physical examination essentially negative. The pelvis was normal, and the blood Wassermann was negative. Pregnancy was uneventful, and spontaneous labor of 6 hours' duration occurred at term. The placenta separated early and was expressed from the vagina. Position L. O. A., and blood loss 50 cc. The child was normal, its weight being 3120 grams and length 50 cm. The puerperium was afebrile, and mother and child were in excellent condition when discharged on the fifteenth day.

Gross examination of the placenta: The placenta is normal externally; the weight is 555 grams, and the dimensions are 20 by 16 by 2.5 cm. The umbilical cord is 42 cm. long and shows nothing unusual. Sections of the placenta show several small white infarcts near the margin.

Microscopical examination: Besides the white infarcts, there are two small tumors situated 2 cm. from the placental edge. The larger is 3 mm. from the fetal surface. It is nearly round and 3 mm. in diameter. The other is about one third as large and is 2 mm. beneath the first. They have essentially the same

structure. Both have a single or double-layered syncytial investment beneath which is a layer of scantily-nucleated, fibrous tissue, the fibres lying parallel to the tumor surface. There are a few vessels in this layer, but at the point sectioned neither they nor any trabeculae are seen passing inward. The stroma consists of fairly numerous blood-filled capillaries separated by cellular connective tissue. The capillary walls consist of single-layered endothelium. Rather large, oval or round cells are found in the connective tissue. About one third of the larger tumor shows a diminished number of capillaries, and here the connective tissue predominates. The villi of the surrounding tissue are normal, as is also the umbilical cord.

As is indicated by the descriptions, the small tumor in Case 1 was not seen on gross examination, but was found microscopically by chance, and the identity of the nodules in Cases 2 and 6 was not certain until microscopic sections were examined. However, the gross appearance of the tumors in Cases 3, 4, and 5 immediately suggested their nature. In none of the sections examined was the frequently described, vascular, attaching pedicle found, but a careful search was not made, as the tumors were desired for museum specimens. However a definite hilus was found in two tumors—Cases 3 and 5.

In no instance was there polyhydramnios, nor were the tumors otherwise of clinical importance, unless the slight prematurity of the child in Case 3 could be considered of significance. Such observations are in accord with the results of the previous study (1), namely: that excessive quantities of amniotic fluid with consequent premature labor and high fetal mortality were associated almost entirely with the large tumors. The small tumors appeared to be of negligible clinical importance.

Inasmuch as six small angiofibromata of the chorion were found in six hundred carefully examined placentas, it is concluded that, in contrast to the rarity of the large tumors, the small ones are not uncommon.

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ON APOCRINE SWEATGLANDS IN WHITE AND NEGRO MEN AND WOMEN[1]

HANS HOMMA, M.D.

From the Department of Pathology, The Johns Hopkins University and Hospital

Since the publication of the papers of Krause (1844) and Robin (1845), it has been known that there are sweatglands in the skin of the axilla which are larger than the common sweatglands in the human skin. Most authors have thought that these axillary glands represented a special type of sweatglands, but the arguments for differentiation which were put forward were not satisfactory. Still, in 1902, Rabl, as well as Lüneburg, stated that the large sweatglands of the axilla were developed from the small ones and that, therefore, they were identical.

The thorough investigations of Schiefferdecker (1922) made it plain that there are two different types of sweatglands, the exocrine, or e-, and the apocrine, or a-, type, which may be traced through the range of mammalian animals up to man. As for the etymology of these words, it may be said that the root "krin" means secreting, as in the more common word *endocrine*.

The exocrine glands produce a fluid which is formed as little droplets within the protoplasm of the epithelial cells of the sweatgland and then is pushed out into the lumen of the gland without any loss of protoplasmic substance. With the apocrine type, however, a part of the secreting cell itself is lost during secretion. This takes place in such a way that at first the straight free margin of the epithelial cell is bulged out towards the lumen of the sweatgland, until a process is formed, which gets narrower and narrower at its base and, finally, is found isolated in the lumen.

The two types of glands differ also in their embryonic development. The exocrine glands start from the free surface of the skin, while the apocrine glands take origin from a hairfollicle. After the forma-

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tion of the gland, it may happen that the aperture of the duct wanders from the hairfollicle to the free surface. On the other hand, the aperture belonging to an exocrine gland never wanders into a hairfollicle.

On account of this characteristic in the origin of the glands, it is plain that apocrine glands can be found only where hairs occur at some time of life. The palms and soles are free from them.

As Schiefferdecker pointed out, the apocrine glands are very numerous in mammalian animals; there are fewer in apes than in the other animals and fewest in man. In man the exocrine type is found scattered all over the body, while the apocrine glands, as a rule, occur only in certain places; in the axilla they form a dense layer of coils; in the external earduct they are also rather densely arranged; they are much more scattered in the areas around the nipple and around the anus.

Schiefferdecker believes that even the various human races may differ from one another in the abundance of their apocrine sweat-glands. His material, however, consisted only of one Australian negro, one Chinaman, two negroes from Kamerun, five German women and seven German men. The small number of non-European subjects, therefore, made further investigation necessary in order to determine whether Schiefferdecker's suggestion is true.

The large number of colored people who come to autopsy in this department has enabled us to deal with this question in so far as negroes and white men are concerned.

All shades of colors between the darkest and lightest brown are found among the negroes in Baltimore. In order to avoid as far as possible getting skin from individuals of mixed races, we chose such bodies for our examinations as showed all the characteristics of the black race—a very dark brown color, broad lips, a broad flat nose and woolly hair. It was, of course, impossible to determine the original African tribe to which the individual belonged. With white men, however, we were able to find out the European country from which they had emigrated in 7 of our 12 cases, as shown in Table I. The material was just large enough to allow us to judge of the differences between the white and colored people generally, but

was much too small to permit of details as to differences within the white or black race.

Before detailing our results we should like to mention the difficulties which exist in differentiating the two types of glands in a given section of adult human skin. The peculiarities mentioned are of little value in this case, as the gland is not always found in the characteristic stage of apocrine secretion; moreover, post mortem typical features quickly disappear. It is unnecessary to say that the aperture of the duct is seldom seen in the section to be diagnosed.

There are, however, some other characteristics which are available for human skin only. One of these is the size of the gland. The large sweatglands of the older authors are now recognized as apocrine glands. There are, however, glands of transition, of which it is difficult to say whether they belong to the large or small ones.

Another characteristic has to do with the iron content of the glands. It was shown in previous work that iron is found very frequently in the cells of the large axillary sweatglands, whereas it was never met with in the exocrine sweatglands. It may be said now that this is typical for all the apocrine glands in negroes and in white men and not only for the apocrine glands of the axilla.

Some help in the diagnosis may be derived from the degree of post-mortem desquamation, since Veil was able to show that the epithelium of the large sweatglands of the axilla becomes separated from its basal membrane much sooner than that of the ordinary or exocrine sweatglands. This observation holds not only for the apocrine sweatglands of the axilla, but for all the apocrine glands of the human body.

All these properties of the sweatglands enabled us in most of the sections to decide beyond doubt the nature of a gland. With a few glands, however, it was impossible to make a diagnosis. These doubtful instances were counted as negative, but this happened so rarely that the figures were not much changed by this failure.

The pieces of skin were taken from the middle of the breast, from halfway between the umbilicus and the symphysis, from the mons veneris and from the region around the anus. These sites were chosen because it was known that a-glands occur there, although seldom. This sparse distribution favors recognition of differences in

frequency. Also, it was easy to obtain skin from these situations without mutilation. The pieces were fixed in alcohol or Zenker's solution, embedded in paraffine and cut in interrupted serial sections so that the sections were about 0.5 mm. distant from one another.

TABLE I

*White men and women.**

AUTOPSY NUMBER	SEX	AGE	MOTHER	FATHER	NUMBER OF SECTIONS OF THE SKIN OF THE											
					Breast			Abdomen			Mons veneris			Circumanal region		
8313	F.	42	—	—	—	—	12	—	—	5	—	—	16			
8329	M.	29	—	—	—	—	13				8	4	—	—	—	10
8359	F.	40	—	—	—	—	12	—	—	13	5	5	11	—	—	10
8561	M.	75	Engl.	Scotch	—	—	10	3	3	9	7	7	6	—	—	12
8661	M.	53	—	—	—	—	12	—	—	12	3	3	9	—	—	13
8725 Gen. Phil. Hosp.	M.	73	Germ.	Germ.	—	—	11	3	3	8	8	3	4	—	—	12
9929	M.	79	Germ.	Germ.	—	—	12	—	—	11	2	0	10	—	—	12
9931	M.	56	?	French	—	—	9	—	—	12	—	—	12	—	—	12
9932	M.	58	Germ.	Irish	—	—	12	—	—	11	—	—	11	—	—	12
9935	M.	47	Germ.	Germ.	—	—	12	—	—	12	—	—	12	2	0	10
9934	F.	70	Danish	Danish	—	—	12	—	—	10	11	11	1	7	2	5
9936	M.	60	—	—	—	—	12	—	—	12	2	0	10	—	—	12
Number of cases							12			11			12			11
Total number of sections							139			121			149			129
Total number of sections in which a-glands were found							0			6			47			9
Total number of sections in which iron was found in a-glands							—			6			33			2
Per cent of sections in which a-glands were found in all							0			5			31.5			7
Per cent of sections in which iron was found in a-glands							—			100			70			23

* The first figure in each column of both tables beneath the words breast, abdomen, mons veneris and circumanal region indicates the number of sections with a-glands, the second one the number of sections with iron in the a-glands and the third the number of sections without a-glands.

By this method, we made fairly sure that the a-glands in the examined pieces did not escape our observation. All the sections were stained for iron by the method of Turnbull and afterwards with alum carmine.

We also cut sections from the skin of the axilla of all the subjects. In the axilla the a-glands are very densely arranged so that many of them can be examined in a single section. Unless a-glands were found in the section, the other pieces of skin were not examined. We excluded, for instance, two cases, those of a girl of thirteen and a boy of fifteen, whose axillary glands had not yet developed. There

TABLE II

*Colored men and women**

AUTOPSY NUMBER	SEX	AGE	NUMBER OF SECTIONS OF THE SKIN OF THE			
			Breast	Abdomen	Mons veneris	Circumanal region.
8318	M.	38		— — 20	25 25 12	— — 15
8346	M.	27				— — 15
8352	F.	36		— — 13	— — 8	— — 7
8377	F.	44	— — 26	— — 29	— — 12	12 9 2
8445	F.	34	1 — 5	— — 12	16 11 2	10 10 —
8456	F.	56	— — 14	6 12	5 — 7	— — 21
8486	F.	33	— — 7	— — 6	3 3 7	9 — —
8490	M.	40	3 2 9	— — 10	15 15 —	9 9 10
8494	F.	28	— — 11	— — 16	7 7 3	14 14 —
8542	F.	34	— — 12	12 12 —	12 12 —	
8546	M.	55?	2 1 11	12 12 2	6 — 6	— — 12
8548	F.	14	— — 8	— — 12	11 3 —	13 7 —
8556	F.	25	— — 11	2 2 8	5 9 2	29 12 1
Number of cases			10	12	12	12
Total number of sections			120	172	160	179
Total number of sections with a-glands			6	32	109	96
Total number of sections in which iron was found			3	26	81	61
Per cent of sections with a-glands in all			5	19	65	54
Per cent of section in which iron was found in a-glands			50	87	74	63

is scarcely any hope of finding a-glands elsewhere, if the skin of the axilla does not show any.

The results are shown in Table I and II.

Of 139 sections of skin of the breast from 12 cases of white men, not one showed a-glands. Of 120 sections of the same region taken from 10 colored people, only six (5 per cent) showed a-glands.

As to the skin of the abdomen, 121 sections from 11 white men and 172 sections from 12 negroes were examined. A-glands were found in 6 sections (5 per cent) from white and in 32 (18.3 per cent) from colored people.

The corresponding figures found for the skin of the mons veneris are as follows: 151 sections from 12 white subjects gave 47 (31.5 per cent) showing a-glands, while of 168 sections from 12 colored people 109 (64 per cent) were positive. We examined 129 sections of the circumanal region from 11 white people and 179 sections of the same region from 12 colored people; only 9 specimens (7 per cent) from white people showed a-glands, while 96 sections from negroes (53 per cent) were positive.

The total number of sections taken from white people is 538; of these, 62 (11.5 per cent) showed a-glands: of 631 sections from negroes a-glands were found in 243 (38.5 per cent).

Among 62 sections with a-glands from white people, 41 (66 per cent) contained iron in the epithelium of the a-glands. Among 243 sections with a-glands in the skin of colored people, 171 (70 per cent) gave a positive Turnbull's reaction.

As to the frequency of a-glands in men and women, the following figures were obtained: among 610 sections from the colored and white men together, 120 (20 per cent) showed a-glands. Among 566 sections from women, 194 (35 per cent) contained a-glands.

Finally, we compared the percentage of sections with a-glands showing a positive Turnbull's reaction in men with their percentage in women. Of 110 sections containing a-glands from 9 men, 87 (63.5 per cent) gave a positive iron reaction. Among 196 sections with a-glands from 10 women, 125 (79 per cent) contained iron.

SUMMARY

The assumption of Schiefferdecker that a-glands were more frequent in negroes than in white people has been proved for the four regions examined; a-glands have been found in the skin of colored three times as often as in that of white people.

We never found a-glands in the skin from the breast of white men; occasionally they occur in the skin from the breast of negroes.

The largest amount of a-glands in the examined regions was found in the skin of the mons veneris; then comes the skin around the anus, then that of the abdomen, and last, that of the breast.

There is no difference between the frequency of iron in the a-glands of white and colored people; nor is there any difference between men women in this respect. In about 70 per cent of the specimens showing a-glands, iron is found in their epithelium. This finding is characteristic for all the apocrine sweatglands of the human body and not only for those of the axilla, as assumed in my former article; since its publication, I have found iron in the epithelium of the a-glands of the external earduct and also of the area around the nipple.

A-glands occur about twice as often in women as in men. This is in accord with the findings of Waelsch, who found the large sweatglands of the axilla better developed in women than in men.

It does not seem probable that the a-glands are reduced in old age, although this might be assumed, since they do not develop until puberty. We found some of them even in the skin of the abdomen of two old white men (Cases 8561 and 8725).

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A CASE OF INFLUENZA BACILLUS ENDOCARDITIS

ELLA HUTZLER OPPENHEIMER

From the Department of Pathology, The Johns Hopkins University, Baltimore

The reviewers of the literature on subacute bacterial endocarditis (5), (6), (10), (15), (16), (17) usually state that Streptococcus viridans is in 95 per cent of the cases the aetiologic factor, while the Pfeiffer bacillus is responsible for most of the other 5 per cent. Libman (11), (12), as well as Horder (7), (8), (9), has reported numerous cases of influenza bacillus endocarditis. Although in their reviews of the subject each of these authors states that this organism produces a typical subacute bacterial endocarditis, not one of their case reports suggests in every detail a typical viridans infection. For instance, a case described by Horder clinically showed irregular fever, clubbing of the fingers and haematuria, but anatomically atypical warty growths were found on the pulmonary valves. In two other cases described by him no nephritis was demonstrable. Libman states that "thus far the glomerular lesions in the coccus cases have not been discovered in the kidneys of the influenzal cases that have come to postmortem examination." Later on he says that glomerulonephritis is "apt to occur with B. influenzae," but gives no cases demonstrating this point. Furthermore, not one of the other authors (1), (2), (3), (4), in reporting cases of influenza bacillus endocarditis, has shown that the symptoms and lesions in this type of case duplicate entirely those of a viridans infection. In fact, Baehr states that with only one exception—an obscure infection without endocarditis—in all the cases he has examined, embolic glomerulonephritis was due to Streptococcus viridans. The other cases reported differed even more completely from the typical picture.

A short summary of the following case of endocarditis due to B. influenzae is therefore given, because the findings suggest very closely, clinically and pathologically, those of a viridans endocarditis. Not until blood cultures revealed the Pfeiffer bacillus did we know that the case was anything but a typical instance of viridans endocarditis, and at autopsy the lesions were quite indistinguishable.

The clinical history, in brief, is that of a young white man of 28, who one and a half years before admission had been refused life insurance because of a "bad heart." Eleven months before admission he had suffered from a cold with severe shooting pains over his left side, suggestive of a cerebral accident. Since then he had lost weight, had felt weak and without energy, and had had recurrences of the pain. There had been slight dyspnoea and orthopnoea for three months. Acute symptoms had begun two weeks before admission, with oedema of the ankles, dark-colored urine, showers of purplish spots over the body, and nightly chills associated with fever.

On physical examination, there were signs of mitral and aortic valvular disease with cardiac decompensation: irregular high fever and chills; clubbed fingers and toes: a widespread petechial eruption; and a huge spleen. The urine contained many red blood cells in addition to albumin and casts. There was a secondary anaemia. The course was steadily downhill, and the patient died nine days after admission to the hospital. Repeated blood cultures had previously been negative, until the one taken the day before death, which showed a growth of B. influenzae.

The autopsy was performed nine hours after death. The positive findings, quite briefly, are as follows: *Body:* Clubbing of all digits and widespread eruption of petechial haemorrhages. *Serous cavities:* Ascites and hydrothorax. The surfaces show numerous petechiae. *Heart:* Greatly enlarged, weighing 800 grams. There is a marked thickening of the aortic and mitral valves with extensive fresh vegetations superimposed on both. The vegetations are opaque, soft and friable. They form a pendulous mass on the aortic valve and extend down the endocardium to the chordae tendineae of the mitral valve, through the back of the mitral valve to the mitral cusps where another small, cauliflower-like mass has been formed. From here they extend to the auricular endocardium just above the valve. Smears of the vegetations show myriads of tiny Gram-negative rods. Bacterial cultures failed to grow this organism, perhaps owing to the time that had elapsed since death. *Lungs:* There are pleural petechiae and a fresh lobular pneumonia. *Spleen:* Enormous, weighing 2200 grams. Its pulp is quite soft and there are numerous infarcts, some quite fresh, others in the process of healing. *Intestines* and *stomach:*

Numerous petechial haemorrhages in the mucosa. *Liver:* Greatly enlarged, weighing 3500 grams. *Kidneys:* Each weighs 500 grams and is enormously enlarged, with a soft and swollen cortex. Petechial hemorrhages are so numerous that they are sometimes confluent over the surfaces, cut sections, pelves and ureters. *Trachea:* There are many petechiae.

Microscopically, the interesting details are: *Heart:* There is a fine scarring of the myocardium. The aortic valve shows a chronic fibrous thickening with some areas of calcification. The vegetations are large thrombotic masses containing myriads of minute Gram-negative rods. *Spleen:* The pulp is very cellular and shows extensive erythrophagocytosis. Infarcts in various stages of healing are seen. *Kidneys:* There is a typical embolic glomerulonephritis. The glomerular capillaries are plugged with tiny hyaline thrombi, and the glomeruli are, as a result, in varying stages of destruction. There are adherent tufts, blood in the glomerular spaces, and hyalinization, while in some places only a scar remains. Tubules contain hyaline and cellular casts. There are some foci of leucocytes and wandering cells in the interstitial tissues. Tiny bacilli in the thrombi in glomerular tufts are easily stained with methylene blue. As it is impossible to differentiate them from the red-staining hyaline thrombus masses in Gram stains, it is evident that the organisms are Gram-negative.

In *summary*, then, the case, while caused by the Pfeiffer bacillus, clinically shows the protracted course, cardiac disease, splenomegaly, haemorrhagic nephritis, eruption, fever and clubbing of the digits with an onset suggestive of a cerebral accident so characteristic of Streptococcus viridans disease. Anatomically, the type of the vegetations on the chronically diseased and thickened valves, the widespread petechiae, infarcts and embolic nephritis fit in detail into the same picture.

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A MODIFIED WHITE BLOOD-CELL TUMOR OF THE RAT[1]

WARREN H. LEWIS, M.D., AND BOTHO BRÜDA, M.D.

From the Department of Embryology, Carnegie Institution of Washington

This spontaneous tumor was found, August, 1925, by Dr. George Walker, to whom we are greatly indebted for a continuous supply of material from his rat colony. The tumor was located on the under surface of the liver and had all the characteristics of a spindle-cell sarcoma, both macroscopically and microscopically. The tumor has been kept going without difficulty by Dr. Walker in successive generations of rats by implanting every two or three weeks small bits of it subcutaneously into healthy young rats. The tumors increase rapidly in size, may become nearly as large as the hosts and kill the animals in from six weeks to two months. They are firm, white, and cellular with a distinct capsule. After they attain the size of a hazelnut the center becomes necrotic. Metastases are very rare. Grossly, the tumor resembles in many respects the famous sarcoma 180 of the mouse.

Many hundreds of cultures have been made both in autoplasma and in plasma from normal animals. Some cultures have been kept alive for four to five months by washing in plasma and adding fresh plasma from tumor animals every two or three days. Such cultures cannot be kept alive for any length of time with normal plasma. Cells usually begin to migrate from the explants in a few hours and often, especially in normal plasma, form a zone during the first day or two from 1 to 2 mm. wide. Liquefaction usually begins during the first day and ultimately forms a complete circle of fluid about the explant which prevents further migration except on the coverglass. Liquefaction is more rapid in normal plasma than in plasma from a tumor animal. After three or four days the cells tend to become spherical and migration stops unless fresh plasma is added.

[1] Received for publication April 17, 1926.

Only one type of cell has been observed in the cultures and it is an epithelioid modification of the mononuclear blood cell. These cells assume a great variety of forms as they migrate out into the clot or spread out on the coverglass. They may be linear, thread-like or spindle-shaped, multipolar, oval, round and thin, or spherical. At first glance the spindle and multipolar forms might readily be taken for connective-tissue or reticular cells and do resemble them superficially. The cells are continually changing form; oval cells may become multipolar and spindle-like or change back to oval or rounded forms. All the forms are characterized by the presence of film-like pseudopodia at the ends of the processes or on the body of the cell, pseudopodia precisely like those found on the mononuclear blood cells and the epithelioid cells and macrophages into which they change in cultures of the blood or of the buffy coat.

With neutral red and janus green the likeness of these tumor cells to the epithelioid cells of blood cultures is very striking. They all have the characteristic granular central area which stains with neutral red, an eccentrically placed nucleus and a peripheral clear zone in which are short rod and granular mitochondria. There may or may not be an intermediate zone of small fat globules. In some of the cultures, more especially in those that were made by replanting the explant a second time in normal plasma, there developed many giant cells of the Langhans type. These giant cells are highly characteristic and are identical with those that develop from the mononuclear-epithelioid cells in blood cultures and in tubercles. In the tumor cultures they arise by fusion of the epithelioid tumor cells. This fusion has actually been observed. It takes from 15 to 20 minutes for the fusion and complete incorporation of a single cell with a giant cell. During this time the nucleus of the newly incorporated cell passes from the periphery to the nuclear region of the giant cell and its granular central area joins that of the larger cell. Giant cells also fuse with giant cells to form large masses.

The crucial test of the fact that these mononuclear-epithelioid cells are the tumor cells was obtained by implanting several cultures without the explant into a rat. These cultures were made with normal plasma and the explants were washed in Locke solution before the culture was made. After a circle of liquefaction had surrounded the

explant, the clot with the immigrated cells was taken from the coverglass with a needle and implanted into the animal. The explant was left behind and only the clot with the pure culture of mononuclear-epithelioid cells was used. From this procedure a typical tumor developed that was like the original. Cultures from it behaved like those from the original and contained only mononuclear epithelioid cells. Since the original diagnosis of this tumor was a spindle-cell sarcoma, it seems not unlikely that some of the other tumors occurring both in mammals and in man may be white blood-cell tumors.

BOOK REVIEWS

Vital Capacity of the Lungs. By J. A. Myers, M.S., Ph.D., M.D. 140 pp. $3.25. (*The Williams & Wilkins Company, Balto., 1925.*)

In this small volume, the first of its kind on vital capacity, Dr. Myers has prepared a handbook for those who would use vital capacity determinations in health or disease. It comes as a most welcome find to anyone who has been endeavoring to recover from the voluminous literature the important facts and conclusions regarding this subject. For here in well arranged chapters, each with a concise summary, are to be found the facts we want regarding the influence of disease upon the vital capacity, the limitations and uses of the test, the measurements and instruments employed, etc. Especially good is the treatment of the perplexing problem of normal vital capacity standards. Shall we use height, weight, or surface area? A collection of carefully prepared tables is included so that the normal figure can be read off at a glance from the desired standard.

The bibliography of over 200 references gives immediate access to the original sources, including authorities from Hutchinson to Peabody. The reviewer would like to recommend this work especially to two groups, first, those who are already using vital capacity determinations in their diagnosis and prognosis of cardiac and pulmonary diseases, and second, to those who are treating such patients but include no spirometer in their already large armamentarium. This volume will show that there is a distinct place waiting for it.

S. McL.

Rational Gland Therapy For Women. By I. Wanless Dickson, M.B.,F.R.C.S. 96 pp. 4/6 net. (*H. K. Lewis & Co., London, 1926.*)

On putting down this interesting book one wishes he could be sure that everything in it were true and that endocrinology were as simple as it is here pictured. Gland therapy would be so satisfactory whereas, unfortunately, with many of us the reverse is the case. Some of Dr. Dickson's theories with regard to the special inter-relations of the different endocrine glands, while alluring, are hardly supported by sufficient experimental or clinical evidence to be very satisfactory.

However, what is most surprising is the author's ideas with regard to the mammary gland. For a few years preceding 1920, an occasional article would appear in the literature in which some therapeutic results apparently due to the taking of mammary gland extract were reported,—in many of these articles the data furnished were rather meagre. Even such articles have since that time almost entirely ceased to be published.

During 1921, Charlton and Lee, on the experimental side, reported some work

done on rats, which strongly indicated that there is no mammary hormone, while Charlton and Rickey, in the same number of "Endocrinology," stated that on the clinical side the effect upon ovarian activity and this upon uterine function by the feeding of mammary gland substance, if it exists at all, is not constant. The pharmacological council of the American Medical Association in December, 1921, on the basis of reports from the leading obstetricians, gynecologists and pharmacologists of the United States, declared "there is no clear cut evidence to show that the administration of mammary gland preparations is of value," and on the basis of the received reports dropped this preparation from "New and Non-official Remedies" in which it had been given a place a short time previously.

In spite of all this, Dr. Dickson is confident that a special mammary hormone does exist and advises the use of preparations of this gland in a number of different conditions. If there were more detailed accounts of his clinical successes with these preparations, his ideas would be given a more sympathetic reception, even though his experiences with this method of treatment have been so different from that of almost all other gynecologists.

Nevertheless, although the author's views concerning gland therapy in women are uniquely enthusiastic and optimistic, this little book contains much of value and can be read with profit by all who have delved sufficiently deeply in the subject to realize that there still is today in endocrinology, as Dr. Barker in 1905 in an editorial on uric acid in like manner stated, some truth and considerable poetry.

L. B.

Immunochemical Studies. Edited by Carl H. Browning. 239 pp. $4.50. (*Wm. Wood & Co., New York, 1925.*)

This volume consists of a collection of articles, on various phases of serological reactions, which have been previously published by Browning and his associates in various journals. The individual articles have been re-arranged where necessary to avoid repetition and an introductory chapter has been added.

The word "immunochemical" appearing in the title is somewhat misleading, since the chemical phases of immunological reactions do not receive particular consideration. "Serological Studies" would be a more appropriate title. The following topics occur in the volume: (1) Antigenic properties of globin and hemoglobin; (2) mature and immature antibodies; (3) the natural hemolysin of ox serum for guinea-pig corpuscles and conglutination; (4) the effect of osmic acid on receptors; (5) heterophile antigen and antibody; (6) opsonic action and (7) complement.

It is not possible in a brief review to go into the details of the observations made and the conclusions arrived at in the various articles of the collection, since the questions considered are really multitudinous. Suffice it to say that the book can be heartily recommended.

C. G. B.

Radiography. A manual of X-ray technique, interpretation and therapy. By CHARLES D. ENFIELD, M.D., F.A.C.P., Roentgenologist to St. Anthony's and Norton Memorial Infirmary, Louisville, Ky. 299 pp. $10.00. (*P. Blakiston's Son & Co., Phila.*)

The author set out to simplify the science of roentgenology for medical men, not roentgenologists, who are finding it necessary to do their own X-ray work, and has admirably accomplished his purpose. Excellent roentgenograms are used as material for illustration, and are splendidly reproduced.

The section on technique is written in a language easily understandable by those entering upon the study of Roentgenology. The section on diagnosis is clear, free from difficult technical terms, and well written. So are the explanations of the physical laws, and the description of the apparatus.

A study of this manual will act as an excellent guide for physicians who do not command an extensive experience in roentgenology.

M. O.

The Evolution of Anatomy, a Short History of Anatomical and Physiological Discovery to Harvey. By CHARLES SINGER, M.D., F.R.C.P. 205 pp. $4.00. (*London, Kegan Paul, Trench, Trubner, and Co., 1925.*)

The task of reviewing this book is undertaken with admitted bias, the result of many profitable and charmed hours in the company of its author and his co-worker, Mrs. Singer; and of hearing much of its contents delivered under the impressive circumstances of a lecture at the Royal College of Physicians.

Leaving to others the field of anecdote and of personal biography, Dr. Singer has succeeded in writing the history of anatomy and physiology, for the general reader, as a history of ideas. It is sufficient praise to say that he has done this without an obscure or a dull page. The strength of the book lies in the author's ability to draw up a clear synopsis of a work like *De Motu Cordis*, freed from the cumbersome ideas necessary in Harvey's time but now irrelevant and confusing. Singer has thus exposed the significance of Aristole's anatomical works, of Galen, of Mondinus, of Vesalius, and of Harvey, as well as of the scores of lesser workers who helped to weld their ideas into the present mass of anatomical-physiological knowledge. The reader completes the book with a compelling sense of the age-long endeavors of the great anatomists.

Such a book is, of course, not intended to say the final word on controverted subjects, and specialists in the history of anatomy may perhaps find here and there a topic for argument. The reviewer, for instance, (as a result of studies most generously aided by Dr. Singer) would venture to grant Constantine the African and the early Salernitans far higher importance in the recovery of learning than Dr. Singer admits. The book is especially strong in those fields which Dr. Singer has made the subjects of his recent investigations, in particular the Greeks and Mondinus. There is also a very just estimate of Vesalius as the fortunate product of Galenism blended with Humanism.

Finally, the professional anatomist must be grateful that Dr. Singer's wide view of medical history has led him to renewed expression of two cardinal facts, first, that anatomy is one of the great border-lands between art and science and that the anatomist must not lose the aesthetic impulse amid the rigors of pure reason; second, that anatomy is in danger of sterility when it abandons the comparative method and neglects experiment.

G. W. C.

A Textbook of Physiology. By WILLIAM D. ZOETHOUT, PH.D. Second edition, 1925. 615 pp. $4.50. (*St. Louis. The C. V. Mosby Co.*)

The author's preface is justified by the text. Perhaps few texts so admirably meet the purpose for which they are designed as does this one. It contains a maximum of physiological data in readily usable form and a minimum of critical discussion of sources, *i.e.*, the author has carefully sifted his material and presents only such facts of physiology as are seemingly well established. The book is, therefore, not so comprehensive as many of the longer texts, but the data it contains are valid in so far as they are presented. Thus the author is enabled to present the main aspects of physiology in a form especially useful to those who have limited time to give to the subject.

J. G. E.

Basal Metabolism in Health and Disease. E. F. DUBOIS. 372 pp. $4.75. (*Lea and Febiger, Phila. and N. Y., 1924.*)

Except that it would be borrowing a title from the author's master, Graham Lusk, this book could well be called the Science of Nutrition, so comprehensive is it in its treatment of the subjects of nutrition and metabolism. In a striking dedication the book is inscribed to Graham Lusk, pupil of Voit, pupil of Liebig, pupil of Gay-Lussac, pupil of Berthollet and LaPlace, pupils of Lavoisier. The author is one of the outstanding heirs to this scientific lineage and the chief translator of their principles into terms of clinical medicine: as such, he is intensely jealous of the tradition of good work and obviously fearful lest simplification of method and widespread application of the study of basal metabolism in disease lead to lower standards of work and misplaced reliance upon laboratory data in diagnosis. The book is scholarly throughout and a complete résumé of scientific basal metabolic work to date, together with a large amount of information concerning the chemistry and physiology of nutrition in general.

It is refreshing to find that a man of the author's scientific attainments is not "above" good writing; that facility and simplicity of expression and even a little humor are not incompatible with science will be established promptly if other writers will only follow his example. The book needs no recommendation beyond the statement that it was written by DuBois of the Russell Sage Institute.

J. T. K., JR.

The Therapy of Puerperal Fever. By PRIVATDOZENT DR. ROBERT KOEHLER. American edition prepared by Hugo Ehrenfest, M.D. 276 pp. $4.00. (*The C. V. Mosby Co., 1925.*)

The volume reflects, as Ehrenfest points out in the preface to the American Edition, the rational and impartial judgment of an experienced physician on the therapy of puerperal infection. And since no work dealing specifically with this problem has been presented to English speaking physicians within the last fifteen years, the present translation seems to be quite justified.

The author analyses the innumerable therapeutic suggestions that have been made within the last two decades, and discusses in detail the local, surgical and medical therapy including chemotherapy. The author is skeptical of surgical interference as a treatment for puerperal infection. With regard to medicinal therapy he reaches the conclusion that in general it seems indifferent whether we employ serum, vaccines, colloid metals or milk injections. All we can expect from them is the effect either of leucostimulation or of the introduction of a foreign protein, the body reactions toward all of these substances apparently being identical.

The reviewer wonders why the viewpoint now in the foreground of our conceptions, namely, that a number of these agents actually stimulate the activity of the reticulo-endothelial system and thereby cause an increase of the immunizing bodies has not been given any consideration. That a stimulation of the clasmatocytes within the parametrium during labor actually takes place, particularly under the stress of infection, has recently been shown. Observations of this kind reveal, at least in part, the subtle biological reactions which occur within the body tending to localize the process and to prevent general involvement.

J. H.

Lectures on Dyspepsia. By ROBERT HUTCHISON. 76 pp. $1.80. (*Edward Arnold & Co., London, 1925; Longmans & Co., New York.*)

This splendid little book is a compilation of lectures given at different times by the author, who states that it is intended for the general practitioner and offers the specialist nothing: however, one does not proceed far in its pages before taking issue with him on this point.

While the various single lectures covering the clinical investigation of dyspepsia, the diagnostic significance of abdominal pain, conditions which simulate dyspepsia, and the organic dyspepsias, set forth valuable facts whose importance is not sufficiently stressed by most authors, Hutchison's description of "the chronic abdomen" is nothing less than a classic and should be read by everyone practising medicine.

The author is a keen observer, evaluating to a nicety between the pertinent and the unimportant, and possesses in addition that elusive gift, a fascinating style. This book is altogether worth while having, and the reviewer recommends that every internist place it upon his library shelves.

E. H. G.

Our Present Knowledge of Heredity. Mayo Foundation Lectures 1923–1924. 250 pp. $2.50. (*Philadelphia, W. B. Saunders Co.*)

This series of six lectures contains four excellent summaries of our knowledge in as many departments of genetics, a condensed report by Dr. Slye of her well-known work on the hereditary tendency to cancer in certain strains of mice, and a statement of the eugenist's point of view by a zoologist whose contributions to genetics have been of considerable value.

The rediscovery of Mendel's law in 1900 bolstered up the then waning belief advanced by Weismann and others that the chromosomes were the bearers of heredity. Dr. Castle reviews the work of the past quarter century which indicates that the chromosomes furnish the only mechanism by which we can understand the inheritance of the Mendelian characters. Although the geneticist fondly hopes eventually to show that all inheritance is Mendelian in its character, it still remains an open question as to whether genus and species inheritance may not be an affair of the cytoplasm, with the chromosomes merely impressing the Mendelian characters upon the general form.

Dr. McClung, the discoverer of the sex chromosome, takes an impartial view in the chapter on sex-determination as to the actual mechanism involved and stands ready to revise his views in the light of additional evidence being brought forth by the study of sex-reversals and endocrine influences.

Now that we no longer consider the inheritance of acquired characters in terms of the arms of blacksmiths' children, light is beginning to filter into this obscure problem. Dr. Detlefsen remains neutral but presents the recent evidence very capably.

Of particular interest to the medical profession will be Professor Wells' lecture on the inheritability of a tendency to the development of cancer. A critical analysis of human statistics upon this matter seems to be of no value in studying this problem, so that we must turn to animal experimentation. Dr. Slye's work seems to point clearly to the transmission of a tendency to cancerous growth as a simple Mendelian recessive among certain of her strains of mice. If the malignancies of mice represent the same pathological processes that we see in the autopsy room, and if the laws of inheritance are the same for mice and men, the conclusion seems forced upon us that resistance to malignancy is an inherited character.

That caution must be observed in the drawing of general conclusions from what we know of the inheritance of Mendelian characters in certain species is obvious. A bit of recent, and as yet unconfirmed, work by Professor Jeffrey of Harvard seems to indicate that the ubiquitous *Drosophila* is a hybrid, in which case many of the generalizations drawn from study of this geneticists' beast of burden would need revision.

The final essay by Michael F. Guyer, on eugenics, is notable for its complete loss of scientific temper, and utter abandon to broad generalisation unsubstantiated by critical evidence. A great deal of quoted opinion is adduced to support the thesis that our government, which stands for (sic) peace, justice, honesty,

protection of life and property and personal freedom, is rapidly degenerating as a result of the disproportionate increase of the non-nordic elements of our population. Restriction of immigration, and an appeal to the better people to multiply more rapidly are advocated by this zoologist. The article proves again that a scientist considering social problems may be an unreliable as a sociologist.

M. B. S.

Cancer of the Rectum. By W. ERNEST MILES, F.R.C.S. 72 pp. 7/6. (*London, Harrison & Sons, Ltd., 1926.*)

This small volume containing the three Lettsomian lectures by Miles delivered in the spring of 1923 deserves to rank as a classic in the literature dealing with cancer of the rectum. In it the author outlines his study of the pathological history of this disease, showing how his experience with recurrence after operation led him to investigate the paths of spread of cancer of the rectum. Upon the basis of this study of the regions of metastasis he gradually developed the operation which he describes as the "abdomino-perineal resection" and which among many surgeons is known as the Miles operation. There is a clear description of the technical procedure involved in this operation, now the accepted method of dealing thoroughly and logically with carcinoma in this region, and following the operative description is a condensed statement of Miles' clinical experience, with a very convincing improvement in the results of treatment. Before the introduction of his method recurrence took place in from 80 to 90 per cent of all patients operated on and the operative mortality was from 11 to 15 per cent. In Miles' more recent group of cases, seen since the war, the operative mortality has been less than 10 per cent, and in those in which more than five years have elapsed since operation the freedom from recurrence up to the present time is over 70 per cent,—a very striking commentary on the advance obtained by this procedure.

The book is well illustrated and well written, marred only by a few typographical errors. If one were inclined to be critical, the statement that liver metastasis does not occur in cases which are locally operable might be brought into question. As a matter of fact, such cases do occur and, while unusual, are by no means rare. In the last topic considered Miles urges immediate colostomy for cases which are not suitable for a radical cure. There will be a difference of opinion on this point, and the arguments of the author, while having force in particular cases, are not entirely convincing as general principles.

On the whole, one cannot give the book higher praise than by saying it represents the best opinions and experiences on this important subject as yet presented.

H. B. S.

BOOKS RECEIVED

Atlas of Diseases of the Skin. By Prof. G. Riehl and Prof. Leo V. Zumbusch. With Diagnostic and Therapeutic Notes, by John B. Ludy, A.M., M.D., Col., Med.O.R.C., U. S. Army. Part I. *Infectious Diseases of the Skin.* With 69 colored illustrations taken from life. Part II. *Inflammatory Diseases of the Skin.* With 65 colored illustrations taken from life. Part III. *Atrophies, New Formations, Hypertrophies.* With 61 colored illustrations taken from life. 1925. 4°. P. Blakiston's Son & Co., Philadelphia. $25.00.

Tumors of the Colon and Rectum. Their Pathology, Diagnosis and treatment. By Jerome M. Lynch, M.D., and Joseph Felsen, M.D. 1925. 4°. 259 pages. Paul B. Hoeber, New York. $12.00.

Muscular Activity. By Archibald Vivian Hill, M.A., Sc.D., F.R.S. 1926. 8°. 115 pages. The Johns Hopkins University School of Medicine, Lectures on the Herter Foundation, Sixteenth Course, 1924. The Williams & Wilkins Company, Baltimore, 1926.

Association of American Physicians. Transactions. Fortieth session, 1925. 8°. 453 pages. Philadelphia.

Public Health Law. A Manual of Law for Sanitarians. By James A. Tobey, M.S., LL.B. With a foreword by Charles V. Chapin, M.D., Sc.D. 1926. 8°. 304 pages. The Williams & Wilkins Company, Baltimore. $4.50.

Light and Health. A Discussion of Light and Other Radiations in Relation to Life and to Health. By M. Luckiesh and A. J. Pacini. Illustrated. 1926. 8°. 302 pages. The Williams & Wilkins Company, Baltimore. $5.00.

Ultra-Violet Rays in the Treatment and Cure of Disease. By Percy Hall, M.R.C.S. (Eng.), L.R.C.P. (Lond.). With introductions by Sir Henry Gauvain, M.A., M.D., M.C. (Camb.) and Leonard E. Hill, M.B. (Lond.), F.R.S. 1924. 8°. 110 pages. C. V. Mosby Company, St. Louis. $3.75.

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New York University and Bellevue Hospital Medical College. Collected reprints from the Department of Experimental Surgery. Volume 4, 1922–1925. New York.

Lactobacillus Acidophilus. By Nicholas Kopeloff, Ph.D. 1926. 8°. 211 pages. The Williams & Wilkins Company, Baltimore. $5.00.

The Medical Society of London. Transactions. Volume the forty-sixth, 1922–23; and volume the forty-seventh, 1924. Printed for the Society, London.

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